Comparison of Three Methods for Determining Fig Endosepsis Caused by *Fusarium moniliforme* and Other Molds in Caprifigs and Calimyrna Figs

THEMIS J. MICHAILIDES, Associate Plant Pathologist, DAVID P. MORGAN, Staff Research Associate, Department of Plant Pathology, University of California, Davis, Kearney Agricultural Center, 9240 S. Riverbend Ave., Parlier 93648; and RON KLAMM, Director, California Fig Institute, 3425 N. First St., Fresno 93726

ABSTRACT

Michailides, T. J., Morgan, D. P., and Klamm, R. 1994. Comparison of three methods for determining fig endosepsis caused by *Fusarium moniliforme* and other molds in caprifigs and Calimyrna figs. Plant Dis. 78:44-50.

Traditionally, the California Fig Institute (CFI), as a service to the California fig industry, has checked levels of fig endosepsis caused by *Fusarium moniliforme* and other molds in inedible caprifig profichi (spring crop of fig pollinator). This was done by scooping out the internal inflorescences plus parts of the inner scales along the ostiolar canal of figs, placing the mixture in a petri dish, then pouring and mixing melted potato-dextrose agar (PDA) with the fruit tissues. This study reports a new technique and compares it with both the conventional scoop-out and the five-scoop techniques used previously. In all tests, the agar-drop and the scoop-out techniques were more efficient than the five-scoop method. In addition, the agar-drop technique was faster than the other two methods and required only 7.5–8 ml of PDA for processing 20 figs, in contrast to the 300 ml required for both the scoop-out and the five-scoop techniques. Based on efficiency, time required, and savings in materials used, the agar-drop technique is the most economical and reliable method and could be used to advantage commercially. In addition, this method is so much simpler that novices can perform it. Using a knife contaminated by cutting a caprifig infected by *F. moniliforme* increased the incidence of fig endosepsis in the caprifigs cut subsequently, a fact which helps explain why the scoop-out method usually revealed higher levels of fig endosepsis in certain experiments.

Additional keywords: *Alternaria alternata, Cladosporium* spp., eye-end rot, *Ficus carica,* internal rot, pink rot, soft rot, syconium

Fig (*Ficus carica* L. cv. Calimyrna) production is a stable and specialized industry found in California and a few other selected areas around the world (7). The Calimyrna cultivar, occupying 85% of the total acreage, is a nonparthenocarpic cultivar, very desirable commercially because the presence of seeds in the fruits contributes to crunchiness in consumer packs and in such baked products as fig newtons. When figs are clean (without internal rot, such as fig endosepsis, smut, Alternaria, Rhizopus, and Penicillium rots, and also free of insects) they are considered healthy, nutritious fruits (6).

In the last 30 years (1961–1991) the acreage of Calimyrna figs has remained very stable (ranging from 2,500 to 4,100 ha) (5). Total hectarage of all bearing varieties, including Calimyrna, is approximately 6,800 ha, with an average annual production over the 5-yr period of 1987–1991 of 49,000 tons of merchantable product amounting to an annual farm value of $15 million (5). Counties with fig orchard hectarage include Fresno, Madera, Merced, and Kings, all located in the San Joaquin Valley in central California. In the last 20 yr, the San Joaquin Valley has become the dominant fig growing area, producing 100% of California's dried figs, which in turn represent 99.9% of U.S. fig production.

Accepted for publication 14 September 1993.

Fig endosepsis (also called brown rot, eye-end rot, pink rot, and soft rot), named for the internal rot caused by *Fusarium moniliforme* J. Sheld., has been a serious disease of figs since 1920 (9). Initially, infected fruit have internal brown spotting on the inflorescences and/or in the pulp. As the disease progresses, the pulp becomes watery; and external symptoms, such as brown water-soaked skin with pink coloration, appear.

In 1927, Caldis (4) discovered that the fig wasp, *Blastophaga psenes* L., was a carrier of fungal spores involved in the rot of fig fruits and identified the main disease as fig endosepsis, naming the pathogen causing it *Fusarium moniliforme* J. Sheld. var. *fici* Caldis. In 1987, Michailides et al (12) found that three species were involved in fig endosepsis, *F. moniliforme, F. solani* (Mart.) Sacc., and *F. episphaeria* (Tode) W.C. Snyder & H.N. Hans. (= *F. dimerum* Penz. in Sacc.) (19). The majority of the isolates were *F. moniliforme,* and comparative studies of several *F. moniliforme* from fig with isolates of *F. moniliforme* from corn indicated that the fig isolates are not host specific. The pathogen was therefore reclassified as *F. moniliforme* after dropping the variety *fici* (18).

At one time in California, the male tree pollinators (caprifigs) were planted in rows or in groups among the Calimyrna trees. However, more than 50 yr ago, Smith and Hansen (16) suggested that caprifigs should be kept as far away as possible from any cultivar of edible figs that needs pollination via fig wasps. Today, new caprifigs are planted in separate orchards, usually far from Calimyrna orchards; and measures have been taken to reduce excess caprifig trees and destroy caprifigs in abandoned orchards (16). Harvesting all of the overwintering (mamme, winter crop) caprifigs, splitting them in half, and dipping them in fungicides reduces endosepsis (16). Once the mamme crop has been treated, it is placed back in the caprifig orchard so that the issuing supposedly "clean" wasps pollinate (caprify) the profichi (spring crop) caprifigs. If caprifig trees are located in or near a Calimyrna orchard, the profichi crop should be removed completely from the trees before wasp emergence to prevent overcaprification, which can lead to an outbreak of endosepsis disease. In late May to early June, depending on the mean daily temperatures, mature profichi fruit (light yellow color) are transferred to the Calimyrna orchards and, depending on the size of the Calimyrna trees, 3–5 (or more) profichi are placed in a paper bag, a plastic net, or a wire-screen basket and stapled on or hung from a main tree scaffold. Female wasps emerging from profichi pass through the staminate flowers (Fig. 1), pick up the pollen on their bodies, and transfer it into the female syconia after entering through the ostiole. Because the flower structure is not suited to oviposition by the wasp, the female cannot lay eggs in the female flowers of the Calimyrna. As she moves from flower to flower trying to oviposit, she involuntarily deposits microconidia of *F. moniliforme* as well as pollen grains attached to her body.

Although several improvements have been made in the original chemical clean-up method (8,14), growers traditionally have continued testing profichi caprifigs by the scooping-out process before using them in the pollination. This method provided a safe and precautionary process for determining the levels of fig endosepsis of profichi (the only crop carrying significant amounts of pollen). Because these figs cannot be treated by being dipped in fungicide solutions, a method that would risk the viability of pollen, growers test the cleanliness of the pro-

fichi crop by collecting random samples of 30–75 fruits and taking them to the California Fig Institute (CFI), where three trained employees scoop out the inflorescences and put them in petri dishes containing potato-dextrose agar (PDA). Because large quantities of profichi samples brought by various growers need immediate processing to make results available to the growers before the beginning of Calimyrna caprification (early June), and because "dirty" lots need to be rechecked, the development of a quick, inexpensive, and efficient method has been needed for some time.

Furthermore, in years such as 1990, when severe frost caused extensive destruction of mamme caprifigs, growers infuse wild caprifigs into their commercial caprifig mamme crop to assure satisfactory caprification. Wild caprifigs are found in locations protected by frost (mainly on the banks of creeks or in areas with water or along the sides of hills) and usually suffer less frost damage. Checking the cleanliness of the wild mamme crop is necessary before using it in commercial orchards.

Checking the cleanliness of the profichi also is necessary to evaluate the success of cleaning procedures used on mamme caprifigs. Unsuccessful mamme treatments result in dirty profichi, and growers who produce dirty profichi usually buy their profichi from growers with clean profichi to ensure a clean crop of edible Calimyrna fruit. Specific instructions referring to ways of sampling, size and number of samples, and time of delivery are given to the growers by the CFI for efficient, proper, and early submission of samples. Early submission of samples is particularly recommended by the CFI since it makes it possible to retest lots when necessary.

The purpose of this research was to develop an efficient, quick, and inexpensive method for determining fig endosepsis and other molds in caprifig fruit crops (mamme, profichi, and mammoni). Parts of this study have been published (11).

MATERIALS AND METHODS

Survey of fig endosepsis in commercial and wild caprifigs. Three commercial caprifig orchards in the San Joaquin Valley were selected, and composite samples of 97–106 caprifigs were collected from 10 trees each of cultivars Stanford, Roeding 3, and Maslin 150, and from wild caprifigs in the proximity of each commercial orchard. In addition, samples were collected from experimental trees in both Yolo (University of California, Davis) and Fresno (University of California, Kearney Agricultural Center, Parlier) counties. Five scoops of inflorescences from each fig were plated in a dish containing acidified (2.5 ml of a 25% [v/v] lactic acid per liter medium) potato-dextrose agar (APDA). Dishes were incubated at 23 ± 1 C, and growth of *F. moniliforme* was recorded 4–5 days later.

Levels of molds in commercial Calimyrna figs and their relationship to caprifig defects. Upon delivery of natural-condition figs by a producer to the dried fig processor, employees of the State Marketing Order, California Dried Fig Advisory Board, supervise the inspection sampling procedure. The entire contents of all containers are dumped for continuous sampling. The samples are then turned over to the Dried Fruit Association of California (DFA) for inspection in accordance with Marketing Order provisions (1,2). Among the defects scored by the DFA inspectors are insect infestation, endosepsis, smut and mold, and sour rot. Smut and endosepsis are easily distinguished from each other, because signs of smut are black, powdery sporulation in the fig cavity, while signs of endosepsis are white or light pink mycelia with powdery sporulation. Averages of defective figs for each crop year and merchantable fig test averages are calculated from analyses of the growers' samples by the California Fig Advisory Board from official inspection certificates of the DFA of California.

Determining fig endosepsis and other molds. All three techniques described below can be used for any type of caprifigs or edible figs.

Conventional CFI scoop-out technique. Caprifigs (mamme, profichi, and mammoni, representing the winter, spring, and summer fruit crops of the male fig pollinator, respectively) and Calimyrna figs were collected from commercial caprifig and Calimyrna orchards. All figs were surface disinfected in 0.08% chlorine solution prepared from a household bleach (containing 5.25% NaOCl) for 3 min and allowed to dry. Figs were split in half and the inflorescences plus all parts of the areas surrounding the ostiolar canal (Fig. 1) were scooped out with a sharp, sterile knife. Care was taken so that pieces of flowers and scales were spread uniformly in each dish. Melted PDA (45–47 C) acidified with lactic acid was poured over the inflorescences, and dishes were swirled and then incubated at 23 ± 1 C for 5–7 days. The presence of colonies of *F. moniliforme* (fig endosepsis) and other fungi, mainly *Cladosporium, Penicillium, Alternaria,* and *Aspergillus* spp. (mold), were recorded.

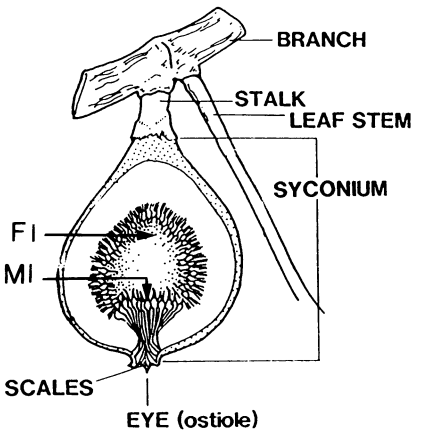

Fig. 1. The fig syconium depicting the various parts of female (FI) and male (MI) inflorescences. FI become seeds in the Calimyrna figs or flower galls in the caprifigs inhabited by the fig wasps; MI develop fully and produce pollen only in the profichi caprifigs.

Five-scoop technique. Caprifigs were prepared as previously described. They were split in half, and a combined total of five scoops (made with sterile tweezers) of small segments of inflorescences were removed from the two halves and plated in a 9-cm-diameter dish containing APDA. Dishes were incubated at 23 ± 1 C for 5–7 days, and fungi developed from the plated inflorescence tissues were recorded.

Agar-drop technique. Caprifigs and Calimyrna figs were collected, surface disinfected in a chlorine solution, and allowed to dry over waxed-wire screens as previously described. To avoid contamination of the inside cavity of mature profichi with open ostioles or mature (amber color) Calimyrna figs, they were surface disinfected by spraying with 70% ethanol. All figs were sectioned with a sharp, sterile knife through the ostiole and forcibly separated into halves (Fig. 2). They were then placed in plastic containers over waxed-wire screens with the sliced surface upward and arranged in five rows each of eight halves per container. Two to five drops of autoclaved (20 min at 121 C) PDA or APDA cooled

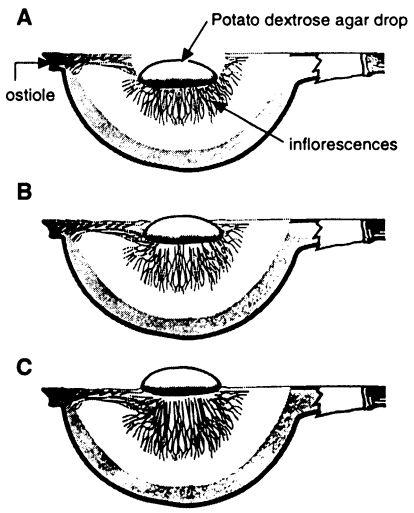

Fig. 2. Different positions of potato-dextrose drops in relationship to the development of the inflorescences of a fig syconium. **(A)** Syconium section with cavity not filled by the inflorescences (large cavity) so that agar drops do not touch all inflorescences. **(B)** Section with cavity partially filled so that agar drops touch the majority of the inflorescences. **(C)** Section with cavity filled by the inflorescences so that agar drops touch only those inflorescences on the surface. (Scale = actual average size of a profichi caprifig.)

to 45 C was placed in the central cavity of each fruit (Fig. 2A–C). Containers were then covered and incubated at 23 ± 1 C for 3–5 days. The incidence of fig endosepsis and mold was determined by viewing each half through a dissecting microscope. In addition, the incidence of fungi present on the fruit ostiole was determined by placing two PDA drops on the ostiole rather than in the cavity.

Comparison of agar-drop with CFI scoop-out techniques. Sixteen lots of profichi caprifigs of 30–74 figs each were collected from commercial caprifig orchards in April 1989. To determine the incidence of fig endosepsis and other molds, caprifigs were split in half, and one half was scooped to remove inflorescences while the other was placed in a plastic container for the agar-drop technique. Petri dishes with the plated fig inflorescences were evaluated for growth of *F. moniliforme* or other filamentous fungi after 5 days incubation at 23 ± 1 C. Figs that had received the agar drops were transported to the laboratory (23 ± 1 C) and observed with a dissecting microscope (10×) after 4–5 days incubation. This experiment was repeated in 1992 with 23 lots of 25 each, 10 lots of 19–22 each, and four lots of 17–25 each of profichi caprifig halves obtained from three commercial growers. The two techniques were compared with LSD using SAS statistics (SAS Institute, Cary, NC).

The agar-drop technique also was used to determine contamination of the fig ostiole by *F. moniliforme* or other fungi (Fig. 1). Figs were surface disinfected, split, and placed in plastic containers over waxed-wire screens, as previously described. Two drops of melted PDA were poured over the ostiole and the surrounding ostiolar scales, figs were incubated at 24 ± 1 C, and the incidence of *F. moniliforme* was determined after 5 days incubation. Four 20-fruit mamme caprifigs were evaluated in 1988, and the experiment was repeated in 1989 and 1992.

Comparison of the agar-drop and five-scoop techniques. To compare these two methods, mammoni and mamme caprifigs were collected on 20 September 1988 and on 20 March 1989, respectively, from a commercial caprifig orchard in Merced County. Mamme from cultivated caprifigs were also collected on 8 February 1989 from a second commercial orchard, and isolated wild caprifigs were collected within a radius of 10 km in the Sierra foothills in Tulare County. The caprifigs were surface disinfected in a 0.08% chlorine solution and used in either the agar-drop or the five-scoop technique as previously described.

To prevent contamination of figs by *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill. in this and all previous experiments, the figs in plastic containers and the APDA dishes with fig tissues were first examined after a 48-hr incubation. When mycelium of *R. stolonifer* was evident (recognized by its fluffy appearance), figs or dishes were sprinkled with dicloran (Botran 75WP), using a salt shaker. The direct contact of the fungicide with some of the hyphae of the fungus restricted growth and prevented nesting and sporulation of *R. stolonifer* to the neighboring figs. The use of the dissecting microscope helped to distinguish when a fig had both Rhizopus and/or endosepsis rots. Abundant production of microconidia by *F. moniliforme* causes a powdery appearance on its hyphae, while the hyphae of *R. stolonifer* are smooth and wider in diameter than those of *F. moniliforme,* allowing correct identification.

Effect of contaminated knife in spreading *F. moniliforme* in caprifigs. To determine whether or not the scoop-out technique could be misleading because of contamination by the knife used for cutting the figs, mamme caprifig fruit of cv. Roeding 3 were collected during March, surface disinfected in a 0.08% chlorine solution as previously described, and cut as follows: A mamme caprifig with golden yellow to brown discoloration (symptoms associated with fig endosepsis, T. J. Michailides, *unpublished*) was cut with a sterilized knife which was then used to cut 13 additional caprifigs. This process was repeated twice to obtain three replications of 26 fig halves, which were placed in three plastic containers (26 fig halves per container), and proc-

Table 1. Survey of fig endosepsis in four commercial caprifig orchards (1987)

Orchard (county)	Cultivar[a]	Caprifigs tested[b]	Incidence of fig endosepsis (%)
(Fresno)	Various selections	120	38
	Wild caprifigs	60	41
1 (Tulare)	Maslin 150	101	27
	Stanford	105	60
	Roeding	102	51
	Wild caprifigs	106	41
2 (Merced)	Stanford	100	23
	Roeding	104	18
	Wild caprifigs	103	42
3 (Merced)	Stanford	97	36
	Roeding	100	54
4 (Yolo)	Stanford (?)[c]	20	24
	Wild caprifigs[c]	20	15

[a] One composite sample of 20 figs each from 10 random trees in Orchards 1, 2, and 3.
[b] Scoops of inflorescences (five-scoop technique) were plated in dishes with acidified potato-dextrose agar and incubated at 24 ± 1 C for 5 days.
[c] Only three trees were sampled.

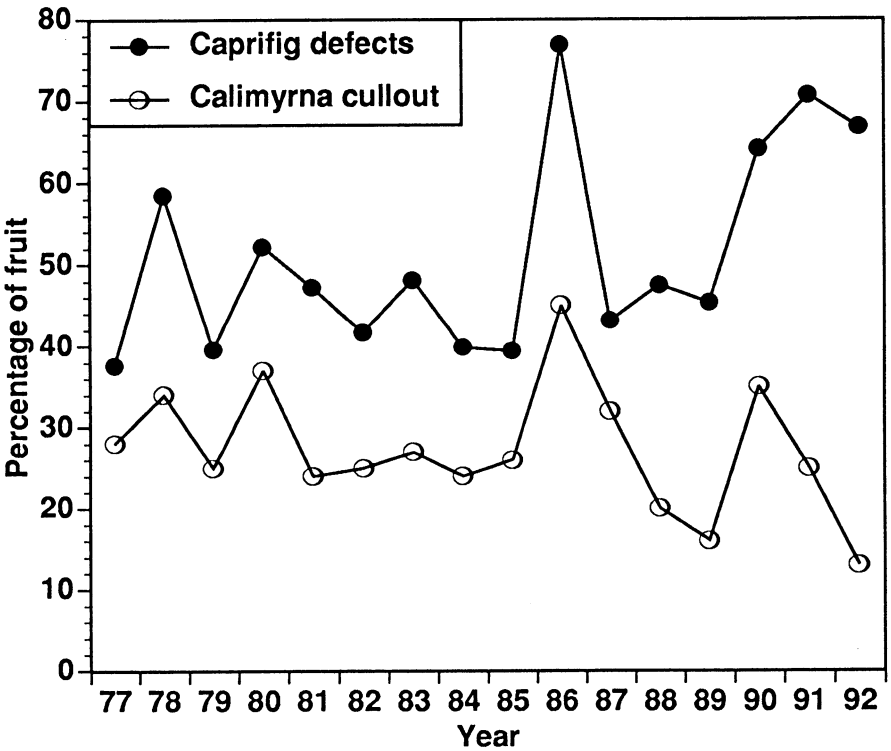

Fig. 3. Relationship of Calimyrna cullout and caprifig defects (include endosepsis and mold) from 1977 to 1992. Values represent yearly averages for the industry and are based on analyses of multiple samples.

essed with the agar-drop technique. The experiment was repeated with mamme caprifig fruits of the late cultivar Stanford collected later in March. Three replications of 26 fig halves each for Roeding 3 and Stanford were used for the agar-drop technique. After sterilization, the same knife was used to cut the caprifigs that were used as controls for the agar-drop technique. Care was taken that the knife not touch the fruit cavity. The incidences of *F. moniliforme* and other fungi were recorded after incubation of the containers for four days at 24 ± 1 C.

RESULTS

Survey of fig endosepsis in commercial and wild caprifigs. This survey, which included three caprifig orchards, indicated that fig endosepsis was endemic in cultivated commercial and wild caprifigs, and that its incidence ranged from 18 to 60% as determined by the five-scoop technique (Table 1). There was no evidence of differences in susceptibility among the various cultivars (Table 1).

Relationship of caprifig cleanliness and Calimyrna cullout. Records kept by the CFI comparing the cleanliness of caprifigs they inspected with cullout of the Calimyrna crop by the DFA show that increased infestation of profichi caprifigs correlated with high Calimyrna cullout (Fig. 3). The data show that 1978, 1980, 1983, 1986, and 1990 were the worst years for Calimyrna cullout and for highest incidence of endosepsis and mold in profichi caprifigs (caprifig defects) used for caprification of Calimyrna figs (Fig. 3). In 1991, there was a high incidence of endosepsis and a low incidence of Calimyrna cullout.

Comparison of the three methods. *Mamme.* In 1988 and 1989, both the agar-drop and the scoop-out methods showed significantly higher levels of fig endosepsis than the five-scoop method (Table 2). However, because different batches of fruit, representing samples from different orchards, have different levels of disease, the levels of fig endosepsis varied considerably between the two tests performed in 1989 (Table 2).

In all tests in both years, 11.3–12.5 min and 7.5–8 ml of PDA were required for processing 20 figs by the agar-drop method contrasted with 30–35 min and 300 ml of PDA necessary with the scoop-out technique (Table 2). The most time-consuming, requiring approximately 44–53 min, was the five-scoop method (Table 2).

In contrast to 1988–1989 results, experiments conducted in 1992 using fewer drops of agar per fig half showed that the agar-drop technique was less efficient in detecting fig endosepsis than the scoop-out technique but more efficient in detecting mold (Table 3). In addition, with the agar-drop technique, only 13–17 min were required to process 20 caprifigs (cutting them, pouring the PDA, and recording fungal colonies developed). In contrast, the scoop-out method took twice to three times as long (28–41 min) (Table 3). Savings in PDA with the agar-drop technique were highly significant (Table 3).

Profichi. Again, the agar-drop technique was quicker and less expensive (considering the amount of PDA required) than the scoop-out technique (Table 4). In the first experiment, the efficiency of the two methods in determining fig endosepsis was similar; but the scoop-out technique was more efficient in determining mold levels. In the second experiment, which used profichi caprifigs harvested 15 days later, some increase in the incidence of fig endosepsis was revealed by both methods; although the scoop-out method tended to show higher efficiency in revealing the disease (Table 4). The time required for cutting the caprifigs, pouring agar, and recording the fungi, and the amounts of PDA required were similar to those in the first experiment. Again, the agar-drop technique was faster and less expensive than the scoop-out (Table 4).

Comparison of the agar-drop technique with the scoop-out used by the CFI. The incidence of fig endosepsis ranged from about 3 to 31.4% with the agar-drop technique and from 3 to 26% with the scoop-out method (Table 5). Nine lots of fruits showed greater, four lower, and three equal incidence of fig endosepsis when the agar-drop technique was used. However, average incidence of fig endosepsis determined was only slightly higher ($P = 0.185$) with the agar-drop than with the scoop-out technique

Table 2. Comparison of three methods for determining incidence of fig endosepsis on mamme caprifigs

Year (experiment) Technique	Incidence of fig endosepsis (%)	Time required to process 20 figs (min:sec)	Potato-dextrose agar used per 20 figs (ml)
1988 (Exp. 1)			
Agar-drop[a]	67.1	12:30	7.5
Scoop-out[b]	51.7	32:30	300.0
5 Scoops/agar dish[b]	46.7	44:00	300.0
LSD ($P < 0.05$)	(9.4)	(3:04)	...
1989 (Exp. 1)			
Agar-drop	89.6	11:42	8.0
Scoop-out	73.3	29:42	300.0
5 Scoops/dish	45.0	49:42	300.0
LSD ($P < 0.05$)	(15.5)	(2:48)	...
1989 (Exp. 2)			
Agar-drop	22.6	11:18	7.5
Scoop-out	26.7	34:36	300.0
5 Scoops/dish	10.0	52:36	300.0
LSD ($P < 0.05$)	(10.0)	(3:12)	...

[a] Each fig received 4–5 drops of melted potato-dextrose agar.
[b] Twenty dishes, each containing 15 ml of agar medium and one fig.

Table 3. Comparison of two methods for determining fig endosepsis on mamme caprifigs (1992)

Experiment Procedure	Endosepsis (%)[a]	Mold (%)[a]	Time required (min:sec)[b] Cut figs	Pour agar	Record fungi	Total time	Potato-dextrose agar used (ml)[a]
Exp. 1							
Scoop-out[c]	75.0	23.3	32:50	3:14	4:07	40:11	300.0
Agar-drop[d]	45.4	32.9	9:18	1:49	5:16	16:23	4.0
LSD ($P < 0.05$)	(7.4)	(6.8)	(2:57)	(0:17)	(0:45)	...	...
Exp. 2							
Scoop-out	76.7	11.7	22:20	2:03	2:47	27:10	300.0
Agar-drop	39.6	24.2	6:40	1:07	4:42	12:29	2.4
LSD ($P < 0.05$)	(9.3)	(6.1)	(1:54)	(0:07)	(0:23)	...	...

[a] Average of six replications of 20 caprifigs each.
[b] Average of six replications.
[c] Each petri dish contained 15 ml of potato-dextrose agar (PDA).
[d] Each fig received 2–3 drops of melted PDA.

Table 4. Comparison of two methods for determining fig endosepsis on profichi caprifigs (1992)

Date (experiment) Method	Endosepsis (%)[a]	Mold (%)[a]	Time required (min:sec)[b] Cut figs	Pour agar	Record fungi	Total time[b]	Potato-dextrose agar used (ml)
19 May/Exp. 1							
Scoop-out[c]	44.2	40.0	18:48	4:05	2:51	25:57 ± 1:15	300.0
Agar-drop[d]	46.3	15.8	5:22	2:35	4:31	12:30 ± 0:53	5.8
LSD ($P < 0.05$)	16.9	6.7	0:43	0:34	0:23	...	...
3 June/Exp. 2							
Scoop-out	67.5	20.8	13:33	3:51	3:44	21:08 ± 0:29	300.0
Agar-drop	59.2	15.0	4:02	1:39	5:49	11:31 ± 0:25	5.3
LSD ($P < 0.05$)	11.9	7.4	0:30	0:17	0:17	...	...

[a] Average of six replications of 20 caprifigs each.
[b] Average of six recordings.
[c] Each petri dish contained 15 ml of potato-dextrose agar (PDA).
[d] Each fig half received 3–4 drops of melted PDA.

Table 5. Comparison of the agar-drop (Kearney Agricultural Center) and scoop-out (California Fig Institute) techniques for detecting fig endosepsis and mold in profichi caprifig (1989)

Lot[a]	Endosepsis (%)[b] Agar-drop	Endosepsis (%)[b] Scoop-out[c]	Mold (%)[b] Agar-drop	Mold (%)[b] Scoop-out[c]
1	2.9	8.6	5.7	20.0
2	11.4	11.4	20.0	37.1
3	23.3	17.1	6.7	34.4
4	16.7	11.4	13.3	28.6
5	20.5	17.1	5.1	42.9
6	20.0	17.1	2.9	20.0
7	28.6	20.0	17.1	45.7
8	20.0	20.0	11.4	25.8
9	31.4	25.7	8.6	45.8
10	5.7	14.3	11.4	45.8
11	22.2	14.3	8.3	34.3
12	8.6	8.6	14.3	22.9
13	5.9	2.9	5.9	8.6
14	6.1	2.9	3.0	40.0
15	5.7	8.6	2.9	14.3
16	22.2	24.4	8.9	26.7
Avg.	15.7	14.0	9.1	30.8*[d]

[a] Lot 3 had 30 figs, lot 5 had 74, lot 6 had 70, lot 11 had 36, lot 13 had 34, lot 14 had 33, and lot 16 had 45; all other lots had 35 figs; all figs were collected on 27–28 April.
[b] Evaluations of endosepsis and mold were by two different people.
[c] Traditionally used by the California Fig Institute.
[d] * = Significant with a t pairwise test at $P < 0.05$.

Table 6. Comparison of the agar-drop and five-scoop techniques in determining incidence of fig endosepsis in winter (mammoni) and spring (mamme) caprifigs

Cultivar and caprifig type[a]	Fig endosepsis (%) Agar-drop method[b]	Fig endosepsis (%) Five-scoop method[c]
1988		
Mammoni	86.4*[d]	81.5
Mamme	45.3*	21.3
Mamme	52.0	47.5
Mamme (Roeding 3)	82.2*	68.6
Mamme (Stanford)	70.0*	46.7
Wild caprifig	25.9*	12.0
1989		
Wild caprifig (SC-1)	67.1	ND[e]
Wild caprifig (Butlr-2)	62.9*	20
Wild caprifig (BM-7)	62.0	ND
Roeding 3	59.6*	26
Stanford	48.8*	26
Maslin 150	45.4*	28
Wild caprifig (Butlr-1)	39.6*	30
Wild caprifig (SC-2)	27.9*	12
Wild caprifig (Woodlake)[f]	10.4	ND

[a] Mammoni were collected on 20 September 1988.
[b] Average of six 20-fruit replicates for the agar-drop method.
[c] Six replicates of 20 acidified potato-dextrose agar dishes containing five scoops per dish.
[d] * = Significant with a t pairwise test at $P < 0.05$.
[e] ND = not determined.
[f] Only three 20-fruit replications were used.

(Table 5). The average incidence of molds was significantly greater ($P < 0.05$) for the scoop-out than for the agar-drop technique (Table 5).

Comparison of the agar-drop and the five-scoop technique (1988, 1989). In both 1988 and 1989, whether using cultivated or wild caprifigs and regardless of the cultivar, the agar-drop technique revealed higher incidence of fig endosepsis than the five-scoop method in most cases (Table 6). However, the incidence of endosepsis varied from sample to sample. For instance, among the samples tested in 1988, the mammoni caprifigs were highly contaminated; while samples of wild caprifigs showed the lowest incidence.

In 1989, endosepsis varied from 10.4 to 67.1% in wild caprifigs and from 45.4 to 59.6% among the cultivated caprifigs, all collected on 8 February 1989 from the Orosi area in Tulare County. In all cases, the agar-drop technique revealed significantly higher incidence of fig endosepsis; while the five-scoop technique was not very efficient (Table 6).

The agar-drop technique also was shown to be a quick and effective method of determining contamination of the ostiole of figs. In two tests, 43–50% and 67–87% of the fig ostioles, respectively, were contaminated with propagules of *F. moniliforme*, and 55–60% and 19–43% with propagules of other fungi. Fungi such as *F. moniliforme*, *Alternaria alternata* (Fr.:Fr.) Keissl., *Botrytis cinerea* (Pers.:Fr.), *R. stolonifer*, *Paecilomyces lilacinus* (Thom) R.A. Sampson, and *Cladosporium* and *Penicillium* spp. could be identified easily on the solidified agar drops. Occasionally, *Gonatobotrys simplex* Corda was associated with either *A. alternata* or a species of *Cladosporium* growing on the ostiole of caprifigs.

Effect of contaminated knife in spreading *F. moniliforme* in caprifigs. Cutting caprifigs with a contaminated knife significantly increased ($P < 0.05$) the incidence of *F. moniliforme* in caprifigs of both cultivars but did not affect the incidence of other molds, which ranged from 33–49% (Fig. 4). Molds included *A. alternata*, *B. cinerea*, *P. lilacinus*, *R.*

stolonifer, Cladosporium and *Penicillium* spp., and various white yeasts.

DISCUSSION

During the period 1986–1991, the California Fig Industry experienced unexplainable increases in levels of diseases (fig endosepsis, smut, and mold [15]) in the Calimyrna crop (5). Our survey of both commercial caprifig orchards and wild caprifigs indicated that fig endosepsis disease was very common, with levels ranging from 18 to 60%. This epidemic caused such concern among growers that in 1987 the CFI started supporting research to help solve the problem. One of the priorities of the industry was to develop a quick, efficient, and inexpensive method for checking the cleanliness of commercial profichi caprifigs.

Although natural media such as those recommended earlier (10,17), nonselective media (3), and selective media (13) are excellent for the isolation of *Fusarium* spp. from plant tissue and soil, this study shows that PDA and APDA are excellent media for isolating *F. moniliforme* from fig fruits. The CFI has for several decades used the tedious, traditional method of checking profichi caprifigs by scooping out inflorescences of the fruit in dishes containing PDA, or recently, APDA. To save time and money we developed the agar-drop technique, compared in this study with both the traditional scoop-out and five-scoop techniques.

The agar-drop technique is simple and inexpensive and as efficient as the scoop-out technique if 7.5–8 ml of PDA are used to process 20 figs. Because both the agar-drop and the scoop-out methods are more accurate than the five-scoop technique, the five-scoop technique should not be used. The higher incidence of fig endosepsis revealed by the agar-drop and scoop-out methods can be explained by the fact that both utilize the majority of the inflorescences, while in the five-scoop method only a few inflorescences are plated, resulting in a greater chance of missing fungal propagules present in the cavity. Although the incidence of fig endosepsis in mamme caprifigs determined by the agar-drop and the scoop-out methods was about the same, the agar-drop technique is two to three times faster and considerably less expensive than either of the other methods. The scoop-out method is significantly more accurate and quicker than the five-scoop technique but requires as much PDA (Table 2). Considering all these criteria, the agar-drop technique is superior and should be used commercially. In fact, the CFI intends to adopt this technique in future checks of commercial profichi and wild mamme caprifigs.

The comparison of the agar-drop with the scoop-out method in mamme caprifigs in 1992 indicated a significantly higher efficiency of the scoop-out technique, suggesting that the efficiency of the method depends also on the stage of fruit development. When fruit are collected 1–2 wk after caprification when the inflorescences are small and the cavity large, the PDA drops come in contact with only a small portion of the inflorescences (Fig. 2A). Similarly, when fruit are at a later stage, the inflorescences fill the fruit cavity, making it difficult for the melted agar drops to reach the majority of inflorescences (Fig. 2C), so that propagules of *F. moniliforme* trapped among the inflorescences may escape. However, by scraping all inflorescences and spreading them in a dish with APDA, hidden *F. moniliforme* propagules come into contact with the agar and grow. In addition, the lower percentages of incidence of fig endosepsis obtained with the agar-drop method in profichi caprifigs in 1992 can be attributed to the use of only two to three drops of PDA, in comparison with the four to five used previously (Table 3). Data on time required to process 20 figs were about the same in the results of 1988, 1989, and 1992 (Tables 2 and 3).

Sampling from 28 April to 20 May and testing of profichi caprifigs by the CFI (scoop-out technique) and by us (agar-drop technique) were equally efficient in detecting fig endosepsis (Table 5). At this developmental stage of profichi, the cavities are partially filled and the PDA agar drops came in contact with the majority of the inflorescences (Fig. 2B). Later (3 June) sampling favored the scoop-out technique (Table 4). These results suggest that in order to obtain reliable results, sampling of profichi should be completed by or before 20 May. Growers with a large acreage of caprifigs try to follow this schedule, although a few collect samples much too early. The agar-drop technique is not recommended for later samplings, because the inflorescences will fill the cavity and the efficiency of this technique will be reduced considerably.

To reach the cavity of the syconium, fig wasps have to go through the ostiolar channel (Fig. 1), a process expected to contaminate both of these fig parts. Interestingly, the agar-drop technique revealed similar microorganisms and about the same incidences of fig endosepsis in both the ostiole and the cavity.

There are several advantages to using the agar-drop technique. 1) Commercial samples can be processed quickly, facilitating the checkup or recheck of profichi to be used for caprification of Calimyrna figs. 2) A quick checkup can be made of wild caprifigs when necessary after a severe frost. 3) Variability among the replicated samples tested is greatly reduced, indicating that the agar-drop technique represents a more reliable method.

In general, levels of molds determined by the CFI using the scoop-out method were higher than those determined using the agar-drop technique, suggesting possible contamination during the process; although in CFI's testing program, separate scalpels are used to cut the caprifig and to remove the inside, and each scalpel is dipped in methanol and flamed before each use. However, no other precautionary measurements were taken to avoid contamination during the performance of the scoop-out technique at the CFI. In contrast, all precautionary measures were taken in our laboratory (wiping the bench with 70% alcohol before covering it with wet cheesecloth, and surface disinfecting caprifigs with chlorinated water to avoid any contamination with external propagules). In our tests, mold contamination did not differ significantly with either method. In addition, since we observe figs with a dissecting microscope, identification of various genera of fungi present is possible. Furthermore, plastic disposable dishes used in the scoop-out and the five-scoop methods represent an additional, irreversible expense. In contrast, the plastic containers and waxed-wire screens used in the agar-drop method can be surface disinfected by dipping in a 0.15% chlorine solution and used again.

In summary, although both the agar-drop and the scoop-out techniques are almost equally effective in determining levels of fig endosepsis when performed at the right developmental stage of caprifigs, the agar-drop technique is preferable since it requires approximately one-third of the time and one-fortieth of the PDA.

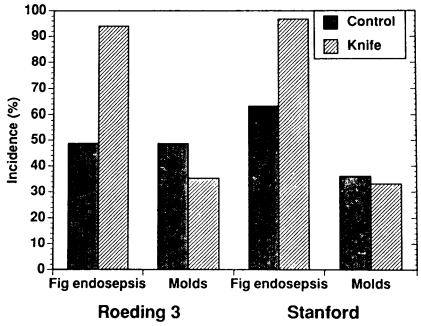

Fig. 4. Effect on spread of fig endosepsis among caprifigs by knife contaminated with *Fusarium moniliforme* being used to cut mamme of Roeding 3 and Stanford caprifig cultivars. (For fig endosepsis, LSD = 13.3 and 23.4%, and for mold 11.0 and 7.4%, for Roeding 3 and Stanford, respectively, at $P < 0.05$.)

ACKNOWLEDGMENTS

We thank William P. Butler (caprifig grower) and Kevin Herman (caprifig and edible fig grower) for providing caprifigs and Calimyrna figs, Andrea Lowe and Dee Hoy for technical assistance, the California Fig Institute for financial support of this project, and Mark A. Doster for reviewing this manuscript.

LITERATURE CITED

1. Anonymous. 1991. Administrative rules and regulations, as amended for the marketing order for dried figs, as amended effective 1 August. California Fig Institute, Fresno.
2. Anonymous. 1992. Amendments to the administrative rules and regulations for the marketing order for dried figs, as amended effective 25 August. California Fig Institute, Fresno.
3. Bonish, P. M., and di Menna, M. E. 1993. A comparison of two methods for isolating *Fusarium* spp. from pasture herbage. Mycol. Res. 97:91-94.
4. Caldis, P. D. 1927. Etiology and transmission of endosepsis (internal rot) of the fruit of the fig. Hilgardia 2(7):288-328.
5. California Fig Industry. 1992. Statistical review of the California fig industry. California Fig Institute and California Fig Advisory Board, Fresno.
6. Duxbury, D. D. 1986. Ingredients spotlight. Food Processing. May 1986.
7. Ferguson, L., Michailides, T. J., and Shorey, H. H. 1990. The California fig industry. Hortic. Rev. 12:409-490.
8. Gerdts, M. H. 1972. Caprifig fungicide experiments. Pages 14-15 in: Proc. Calif. Fig Inst. Res.
9. Hansen, H. N. 1928. Endosepsis and its control in caprifigs. Phytopathology 18:931-938.
10. Hansen, H. N., and Snyder, W. C. 1947. Gaseous sterilization of biological materials for use as culture media. Phytopathology 37:369-371.
11. Michailides, T. J., Morgan, D. P., and Lowe, A. 1990. Comparison of three methods for determining fig endosepsis or other molds in mamme caprifigs. Fig Res. Rep. Crop Yr. 1989. California Fig Industry, Fresno.
12. Michailides, T. J., Ogawa, J. M., and Ferguson, L. 1987. Investigations on the correlation of fig endosepsis on Calimyrna fig with caprifig infestations by *Fusarium moniliforme*. Annu. Res. Rep. Crop Yr. 1987. California Fig Institute, Fresno.
13. Nelson, P. E., Toussoun, T. A., Burgess, L. W., Marasas, W. F. O., and Liddell, C. M. 1986. Isolating, identifying, and producing inoculum of pathogenic species of *Fusarium*. Pages 54-59 in: Methods for Evaluating Pesticides for Control of Plant Pathogens. K. D. Hickey, ed. American Phytopathological Society, St. Paul, MN.
14. Obenauf, G. L., Ogawa, J. M., Lee, K., and Frate, C. A. 1982. Fungicide control of molds that attack caprifigs. Plant Dis. 66:566-567.
15. Smith, R. E., and Hansen, H. N. 1931. Fruit spoilage diseases of figs. Univ. Calif. Exp. Stn. Bull. No. 506.
16. Smith, R. E., and Hansen, H. N. 1935. Directions for control of endosepsis in figs. 1934-1935. Univ. Calif. Agric. Exp. Stn.
17. Snyder, W. C., and Hansen, H. N. 1947. Advantages of natural media and environments in the culture of fungi. Phytopathology 37:420-421.
18. Subbarao, K. V., and Michailides, T. J. 1992. Re-evaluation of *Fusarium moniliforme*, the causal agent of fig endosepsis. Mycol. Res. 96:766-768.
19. Subbarao, K. V., and Michailides, T. J. 1993. Virulence of *Fusarium* species causing fig endosepsis in cultivated and wild caprifigs. Phytopathology 83:527-533.